The taxonomic significance of anatomical characters of the leaf in the southern African species of *Canthium s.l.* (Rubiaceae)

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Fourteen species of *Canthium s.l.* recorded in southern Africa were studied. The anatomy of the leaf is described and the taxonomic and diagnostic value of the characters discussed. As with the stem, several anatomical features of the leaf are useful in distinguishing between taxa. These include the structure of the hairs, the thickness of the lamina, the shape of the midrib area, the thickness of the cuticle, the shape of the epidermal cells, the distribution of stomata, the extent and pattern of cutinization of the epidermal cells, the composition of the mesophyll including the abundance and distribution pattern of tanniniferous cells and the shape of the collenchyma in the midrib area. With regard to relationships between species not necessarily confined to southern Africa, indications are that leaf anatomical features may be of value. It also appears that leaf anatomy may be useful for diagnostic purposes irrespective of the stage of development of the leaf. On the basis of leaf anatomy three distinct groups were recognized, corresponding with the recently proposed segregate genera *Canthium* Lam. *s.str.* (in a southern African context), *Psydrax* Gaertn. and *Keetia* Phill. Nearly all species are anatomically homogeneous and all species could be distinguished from one another.

Hierdie ondersoek is op 14 spesies van *Canthium s.l.* wat in Suider-Afrika voorkom, gebaseer. Die anatomie van die blaar word beskryf en die taksonomiese en diagnostiese waarde van die kenmerke bespreek. Soos in die geval met die stingel is vasgestel dat verskeie anatomiese kenmerke van die blaar van waarde is om tussen taksons te onderskei. Dit sluit in die struktuur van die hare, dikte van die lamina, die vorm van die hoofaargebied, dikte van die kutikula, vorm van die epidermale selle, verspreiding van stomas, mate en patroon van kutinisering van epidermale selle, samestelling van die mesofil insluitende die voorkoms en verspreidingspatroon van tannienhoudende selle en vorm van die kollenchiem in die hoofaargebied. Aanduidings bestaan dat blaaranatomiese kenmerke van waarde is by die oorweging van die verwantskappe tussen spesies wat nie noodwendig tot Suider-Afrika beperk is nie. Dit blyk ook dat blaaranatomie ongeag die stadium van blaarontwikkeling waardevol vir diagnostiese doeleindes is. Op grond van die blaaranatomie is drie erkenbare groepe geïdentifiseer wat ooreenstem met die onlangse voorstel van drie afsonderlike genusse, *Canthium* Lam. *s.str.* (in 'n Suider-Afrika-verband), *Psydrax* Gaertn. en *Keetia* Phill. Anatomies vertoon bykans al die spesies min infraspesifieke variasie en die meeste kan van mekaar onderskei word.

Keywords: Anatomy, *Canthium, Keetia*, leaf, *Psydrax*

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Introduction

Difficulties exist with the delimitation of genera within the Vanguerieae, of which *Canthium* Lam. *s.l.* is a notable example. Bridson (1985, 1986) has reinstated the genera *Psydrax* Gaertn. and *Keetia* Phill., retaining the genus *Canthium* in a narrow sense. Problems experienced with the southern African species of *Canthium s.l.* at various levels of the taxonomic hierarchy are discussed by Tilney (1986). Bridson's above proposals, based mainly on morphological evidence, of changing the generic limits of the African taxa included in *Canthium s.l.*, are supported by an anatomical and morphological (pollen, fruit and seed) study of the southern African representatives (Tilney 1986). Tilney *et al.* (1988) have shown that some of the uncertainty surrounding the generic and specific limits in *Canthium s.l.* can be resolved using characters of the young stem. Principal differentiating anatomical features of *Canthium s.str., Psydrax* and *Keetia* include: structure of the hairs, range in cuticle thickness, shape of epidermal cells, relative abundance of two types of pericyclic fibres, position of initiation of the periderm and the shape of the vascular cylinder and pith.

Robbrecht & Puff (1986) found that the available leaf anatomical data on a group of genera of the Gardenieae and related tribes 'do not seem to provide taxonomically significant classification characters'. However, Puff *et al.* (1984) record several leaf anatomical characters that differ between species of *Alberta* E. Mey. and *Nematostylis* Hook. f. Little use appears to have been made of such characters for problems relating to *Canthium s.l.* However, reference is made in certain anatomical investigations to a limited number of *Canthium* species. These include work by Gerhard (1902), Solereder (1908) and Metcalfe & Chalk (1950, 1979). The findings of these researchers are included in a more detailed evaluation of various characters in Tilney (1986).

Leaf anatomical features provide further support for Bridson's (1985, 1986) proposals and, in some cases, are diagnostically useful at the specific and subspecific levels. In this paper, some of the characters of the leaf are evaluated and their taxonomic significance is discussed.

Table 1 Species of *Canthium s.l.* studied, with voucher specimens, or grouped according to Bridson's (pers. comm., 1985, 1986) proposal of three separate genera. Explanation of symbols: a = taxon; b = collector; c = collecter's number; d = grid reference (if known) for collecting locality (Edwards & Leistner 1971); e = herbarium acronym; * = rehydrated material (absence of * indicates fresh material); brackets denote material from the same collection

a	b	c	d	e
Canthium* Lam. *s.str.				
C. ciliatum (Eckl. & Zeyh.) Kuntze	Abbott	642*	3130 AA	PRU
		775	3030 CC	PRU
		1050*	3030 CC	PRU
		1051*	3030 CC	PRU
		1435	3030 CC	PRU
	Galpin	8123*	3226 BA	PRE
	Gemmell	s.n.*	2828 CA	PRE
	Goosens	820*	2827 DA	PRE
	Kemp	1452*	2631 AC	PRE
		2893*	3226 DD	PRE
	Kok	724*	2430 AA	PRU
		727	2430 AA	PRU
		730	2430 AA	PRU
	Kotze	813*	2827 DD	PRE
	Rogers	21990*	2430 AA	PRE
	Tilney	183	2430 DC	PRU
		186	2430 DC	PRU
	Van Niekerk	25270*	3326 BC	PRE
	Van Wyk	2731	2230 CD	PRU
		3299*	3228 CB	PRU
		5221*	2229 DD	PRU
	Van Wyk & Theron	4908*	2230 CD	PRU
C. gilfillanii (N.E. Br.) Miller	Tilney	[1	2628 AA	PRU
		1*	2628 AA	PRU
		3	2628 AA	PRU
		4	2628 AA	PRU
		5	2628 AA	PRU
		7	2528 BC	PRU
		15*	2527 CD	PRU
		33*	2529 CB	PRU
		54*	2828 DB	PRU
		84*	2628 AD	PRU
		136	2628 AA	PRU
		138	2628 AA	PRU
		145	2628 AA	PRU
	Verdoorn	467*	2528 CC	PRE
C. inerme (L.f.) Kuntze	Abbott	889	3130 AA	PRU
	Kok	723	2430 AA	PRU
		732	2430 AA	PRU
	Tilney	119	3325 DC	PRU
	Van Wyk & Kok	5794	3030 CC	PRU
		5812	3030 CC	PRU
C. mundianum Cham. & Schlechtd.	Acocks	12141*	3226 DB	PRE
	Kok	717	2330 CA	PRU
	Tilney	2	2628 AA	PRU
		57*	2628 AD	PRU
		60*	2628 AD	PRU
		135	2628 AA	PRU
	Van Wyk & Kok	5777	3030 CA	PRU
C. pauciflorum (Eckl. & Zeyh.) Kuntze	De Winter	9339*	2430 DD	PRE
	Flanagan	2718*	3127 DB	PRE
	Killick & Marais	2145*	2830 AA	PRE
	Kluge	996*	2430 DC	PRE
	Tilney	188	2430 DC	PRU
	Van Jaarsveld	3729*	3226 DB	PRE
C. setiflorum Hiern	Puff	820909-1/1	Ethiopia	WU
		821213-2/8	Ethiopia	WU
		840213-1/4	Inhaca	WU
	Van der Schijff	3562*	2231 CA	PRE
	Van Rooyen	1083*	2231 DB	PRU
		1181*	2231 AC	PRU
	Van Wyk	3734*	2230 DB	PRU
		5951a*	2230 CB	PRU
	Van Wyk & Theron	4953*	2230 CD	PRU
	Ward	3994*	2732 CA	PRE
C. spinosum (Eckl. & Zeyh.) Kuntze	Abbott	1422	3030 CC	PRU
	Dyer	43*	3326 AD	PRE
	Hutchinson	1535*	3326 AD	PRE
	Olivier	1386*	3129 CD	PRU
	Theron	1584*	3129 DA	PRE
	Tilney	120	3325 DC	PRU
C. suberosum Codd	Abbott	933	3130 AA	PRU
		1301	3030 CC	PRU
		1370	3030 CC	PRU
		1468	3030 CC	PRU
	Botha	2065*	2527 DC	PRE
	Compton	28157*	2631 CD	PRE
	Gerstner	5346*	2429 BA	PRE
	Schrire, Van Wyk & Abbott	1803	3129 BD	PRU
	Tilney	14	2527 CD	PRU
		[141	2527 CD	PRU
		141*	2527 CD	PRU
	Van Wyk	5865	3129 BD	PRU
C. vanwykii Tilney & Kok	Abbott	1371	3030 CB	PRU
	Schrire, Van Wyk & Abbott	1792	3129 DA	PRU
	Tilney	[152	3030 CC	PRU
		152*	3030 CC	PRU
	Van Wyk	4189*	3030 CA	PRE
		5110*	3030 CD	PRE
		5316*	3030 CD	PRU
***Keetia* Phill.**				
K. gueinzii (Sond.) Bridson	Abbott	70*	3030 CC	PRU
	Compton	31551*	2531 CD	PRE
	Hafström & Acocks	1929*	3030 CB	PRE
	Kok	712	2330 CA	PRU
	Van Wyk	5888	3129 BC	PRU
***Psydrax* Gaertn.**				
P. fragrantissima (K.Schum.) Bridson	Edwards	2962*	2732 AA	PRE
	Gerstner	4811*	2832 AB	PRE
	Moll	5645*	2732 CB	PRE

Table 1 Continued

a	b	c	d	e
	Nicholson	1165*	2732 AB	PRE
	Ross	2362*	2632 CD	PRE
P. livida (Hiern)	Galpin	13789*	2428 CB	PRE
Bridson	Kok	734	2430 AA	PRU
	Mogg	24534*	2329 AA	PRE
	Pienaar	823*	2528 BC	PRE
	Tilney	34	2528 CA	PRU
P. locuples (K.Schum.)	Edwards	1649*	2931 AB	PRE
Bridson	Moll	4367*	2632 DC	PRE
	Van Wyk	3671*	2230 DB	PRU
		3909*	2230 DA	PRU
		4767*	2230 CD	PRU
		5602*	2230 DA	PRU
		5941*	2230 CB	PRU
P. obovata (Eckl.	Abbott	57*	3130 AA	PRU
& Zeyh.) Bridson	Barnard & Mogg	764*	2530 AB	PRE
	Gerstner	5332*	2429 CA	PRE
	Kok	729	2430 AA	PRU
	Puff	840210-1/4	Inhaca	WU
	Schrire, Van Wyk & Abbott	1800	3129 BD	PRU
	Theron	3663*	2430 DC	PRU
	Tilney	131	3326 DA	PRU
	Van Wyk	A166	3030 CA	PRU
		2577*	2831 DB	PRU
		5331	3030 CC	PRU

Materials, Methods and Terminology

Anatomical features of the leaf were studied in all the southern African species of *Canthium s.l.*, including taxa of doubtful identity. These are listed, together with voucher specimens numbers and provenances, in Table 1.

Small segments of mature laminas were cut from the midrib area at approximately one third of the distance between the junction with the petiole and the leaf apex. Similarly, additional portions of the lamina were cut at the same distance from the petiole and about halfway between the midrib and the margin. For a few species, laminas at various developmental stages were also sectioned to check the constancy of characters (see further in text). For convenience, the term 'leaf blade' is used to mean the area excluding the midrib area although we are aware that the midrib area is part of it. The middle portion of the petiole was selected for study.

The leaf material was prepared for microscopic viewing as described by Tilney *et al.* (1988) for stem material. In addition to the permanent slides, hand-cut sections were made. To investigate the structure of the hairs, peels of mature leaves were examined.

For each specimen, line drawings of the midrib area and a portion of the adjacent leaf blade were prepared with the aid of a projection microscope. Measurements of the thickness of the midrib area and leaf blade, and of the cuticle on the upper and lower sides of both the midrib area and leaf blade and on the upper side of the petiole were made. The ratio of the thickness of the midrib area to the thickness of the leaf blade was calculated. For each species the range of values obtained as well as the average are depicted graphically.

To compare the degree of development of the xylem in the main vascular bundle of the midrib area and in the petiole of the various species, the number of radial tiers of vessels was counted and, for the petiole, the maximum number of vessels per radial tier was noted as well. Detailed descriptions of each species and of *Canthium s.l.* are given by Tilney (1986). Descriptors

Table 2 The principal diagnostic leaf anatomical features of the southern African groups of species of *Canthium s.l.*

	Canthium s.str. group	*Psydrax* group	*Keetia* group
Hairs	present or absent	present (at least on petiole)	present
	septate	non-septate	septate
	fewer than about 10 compartments in largest hairs	–	at least about 15 compartments in largest hairs
	tanniniferous deposits present or absent	tanniniferous deposits absent	tanniniferous deposits present
Shape of main vascular bundle	arc without invaginated ends	arc without invaginated ends	arc with invaginated ends
Distribution/shape of collenchyma above and below main vascular bundle	usually concentrated subepidermally or extending between epidermis and vascular bundle	hourglass shaped	± concentrated subepidermally
Cuticle thickness over lower epidermis of leaf blade	not exceeding about 5 μm[1]	at least 3 μm thick	not exceeding about 3 μm
Shape of outer tangential wall of petiolar epidermal cells	not papillate	papillate	convex
Differentiation of mesophyll into palisade and spongy parenchyma	usually distinct	indistinct	distinct

[1]See discussion of *C. suberosum*

used to indicate abundance and frequency are based on Schmid (1982).

As in the stem anatomical study (Tilney *et al.* 1988), two types of tanniniferous cells were recognized, designated types A and B. The contents of the A-type cells do not have a 'cracked' appearance which is evident in the B-type cells. The 'cracks' in B-type cells are due to physical damage caused during sectioning of the embedded material. It probably signifies chemical peculiarities of the tanniniferous contents of these cells. The inability of the glycol methacrylate monomer mixture (Tilney *et al.* 1988) to infiltrate the cells during embedding, results in a lack of support in polymerized material and the subsequent appearance of cracks when cut on an ultramicrotome. Since the tanniniferous cells appeared to be so useful for taxonomic purposes in the present study, their form in fresh and rehydrated material was compared. Fresh and rehydrated leaves from single specimens of *C. gilfillanii, C. suberosum* and *C. vanwykii* were used, and the appearance of the tanniniferous cells after the two treatments was compared. Since the anatomy of the mature leaves, particularly the pattern of distribution of the tanniniferous cells, appeared to be of diagnostic value, it was decided to compare the anatomy of mature and immature leaves from the same plant to investigate the possible reliability of these characters irrespective of leaf age. For this purpose, *C. gilfillanii* and *C. suberosum* were selected, the former being an essentially non-tanniniferous species except for the midrib area, and the latter highly tanniniferous when mature. With both species, the young leaves selected were less than one third the size of the mature leaves. The results are discussed under the respective species.

The present study shows that anatomical characters of the leaf have much taxonomic value. It was therefore desirable to extend the study to ascertain whether those features found to be distinguishing in the southern

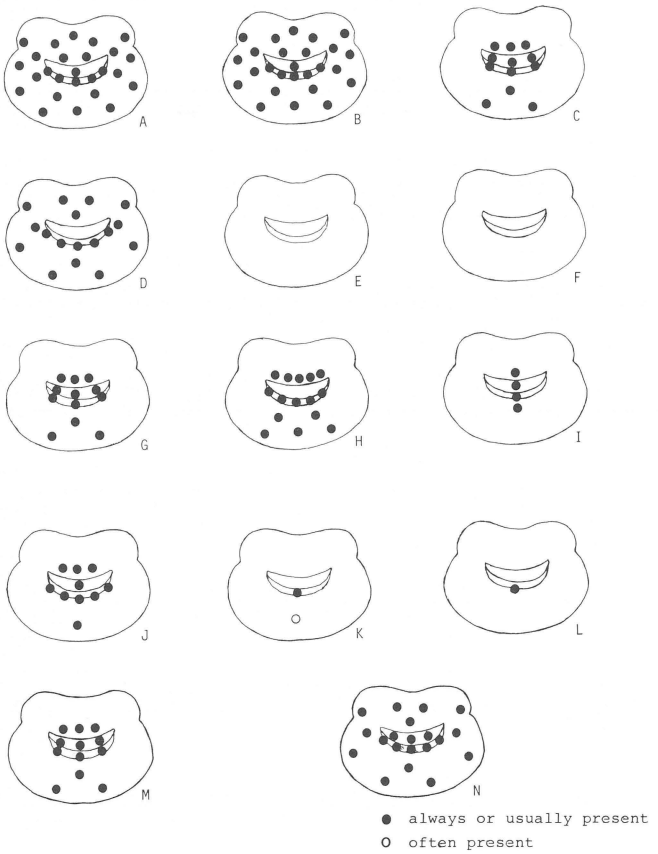


Figure 1 Diagrammatic representation of the distribution pattern of type-B tanniniferous cells in the petiole of the various species of *Canthium s.l.* (A) *C. inerme*; (B) *C. suberosum*; (C) *C. pauciflorum*; (D) *C. setiflorum*; (E) *C. ciliatum*; (F) *C. vanwykii*; (G) *C. gilfillanii*; (H) *C. mundianum*; (I) *C. spinosum*; (J) *Psydrax fragrantissima*; (K) *P. livida*; (L) *P. locuples*; (M) *P. obovata*; (N) *Keetia gueinzii*. The number of dots in a specific tissue denotes the abundance of tanniniferous cells, namely 1 = small numbers, 3 = varying numbers, 5 = abundant.

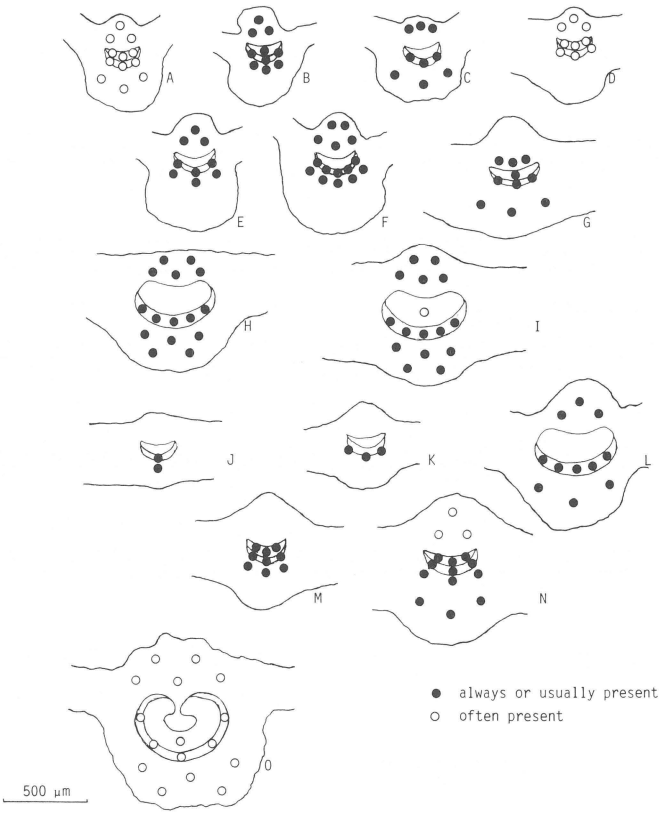


Figure 2 Diagrams of the various species of *Canthium s.l.* showing the outline of transections through the midrib area, drawn from representative specimens on which is superimposed the generalized distribution pattern of the type-B tanniniferous cells based on all the anatomical preparations. (A) *C. ciliatum (Tilney 183)*; (B) *C. spinosum (Tilney 120)*; (C) *C. setiflorum (Van Rooyen 1181)*; (D) *C. vanwykii (Van Wyk 4189)*; (E) *C. gilfillanii (Tilney 3)*; (F) *C. mundianum (Kok 717)*; (G) *C. pauciflorum (Tilney 188)*; (H) *C. inerme (Abbott 889)*; (I) *C. suberosum (Abbott 933)*; (J) *Psydrax fragrantissima (Edwards 2962)*; (K) *P. locuples (Van Wyk 5941)*; (L) *P. livida (Kok 734)*; (M) *P. obovata* group A (*Theron 3663*); (N) *P. obovata* group B (*Van Wyk A166*); (O) *Keetia gueinzii (Abbott 70)*. The number of dots in a specific tissue denotes the abundance of tanniniferous cells, namely 1 = small numbers, 3 = varying numbers, 5 = abundant.

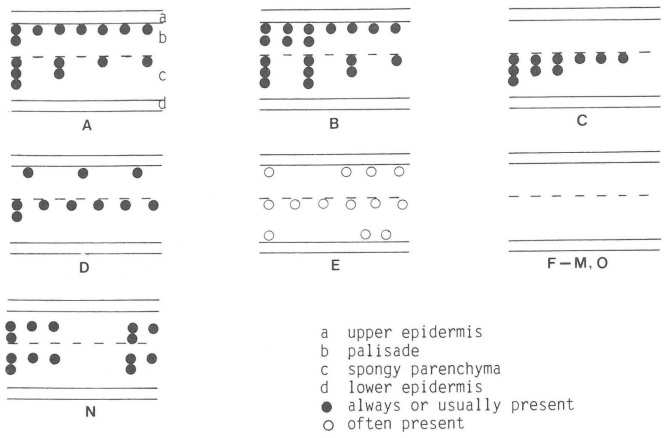


Figure 3 Diagrammatic representation of the distribution pattern of type-B tanniniferous cells in the leaf blade of the various species of *Canthium s.l.* (A) *C. inerme*; (B) *C. suberosum*; (C) *C. pauciflorum*; (D) *C. setiflorum*; (E) *C. ciliatum*; (F) *C. vanwykii*; (G) *C. gilfillanii*; (H) *C. mundianum*; (I) *C. spinosum*; (J) *Psydrax fragrantiisima*; (K) *P. livida*; (L) *P. locuples*; (M) *P. obovata* group A: (N) *P. obovata* group B; (O) *Keetia gueinzii*.

African members were also present in, and diagnostic for, the species occurring in other parts of Africa. Unfortunately an extensive investigation was beyond the scope of this study. However, five specimens were studied, three from Ethiopia and two from Inhaca, Mozambique (Table 1), representing *P. obovata, C. setiflorum* and a specimen tentatively identified as *Keetia gueinzii*. The results of this survey are discussed with the respective species.

Results and Discussion

The southern African species of *Canthium s.l.* could be divided into three distinct groups based on leaf anatomical characters. These groups correspond to the genera *Canthium s.str., Psydrax* and *Keetia* as proposed by Bridson (1985, 1986). Some of the most outstanding taxonomic characters of each group are summarized in Table 2. See also Figures 1 to 5. The leaf anatomical features of each segregate genus are treated separately below.

1. *Canthium s.str.*

Leaf anatomical key to the species of *Canthium s.str.* (excluding petiole)

1a Tanniniferous cells present in the leaf blade, not confined to certain areas.

2a Tanniniferous cells of type-B in 1 or 2 more or less continuous layers adjacent to upper epidermis and in 1–3 layers in middle of spongy parenchyma, very rarely associated with lower epidermis and then in very small numbers; hairs always absent.

3a Midrib area broadly and slightly raised above and very broadly and very conspicuously projecting below; cuticle on lower epidermis of leaf blade about 1 μm thick; outer periclinal cell walls of both epidermal layers of leaf blade and midrib area nearly always slightly cutinized, with minute striae in the midrib area ..... **(a) *C. inerme***

3b Midrib area broadly and conspicuously raised above and below; cuticle on lower epidermis of leaf blade at least about 3 μm thick; outer periclinal cell walls of both epidermal layers of leaf blade and midrib area considerably cutinized, with prominent striae in the midrib area ..... **(b) *C. suberosum***

2b Tanniniferous cells of type-B, if present, very rarely arranged as in 2a; if so, hairs always present.

4a Tanniniferous cells in (1)2(3) rows in spongy parenchyma in middle of mesophyll, absent from the palisade, never associated with lower epidermis; midrib area conspicuously raised above and broadly raised below; hairs absent ..... **(c) *C. pauciflorum***

4b Tanniniferous cells usually in palisade and spongy parenchyma, may be adjacent to lower epidermis; shape of midrib area various but not as above; hairs usually present.

5a Hairs always present, tanniniferous; spongy parenchyma essentially compact .... **(d) *C. setiflorum***

5b Hairs often present, not tanniniferous; spongy parenchyma essentially loosely arranged.

6a Shape of midrib area variable; hairs not confined to midrib area and larger secondary veins; outer periclinal cell walls of epidermal cells of leaf blade usually slightly to very slightly cutinized ..... **(e) *C. ciliatum***

6b Midrib area conspicuously raised above and conspicuously but more broadly projecting below (Figure 2D); hairs confined to midrib area and larger secondary veins; outer periclinal cell walls of epidermal cells of leaf blade considerably cutinized ..... **(f) *C. vanwykii***

1b Tanniniferous cells nearly always absent in the leaf blade or only associated with veins, very rarely in spongy parenchyma where they occasionally form short rows.

7a Spongy parenchyma cells nearly always compact, and storied; mesophyll rarely distinctly differentiated into palisade and spongy parenchyma; druse crystals occasionally present in midrib area (then usually in relatively small numbers) ..... **(g) *C. mundianum, C. gilfillanii***

7b Spongy parenchyma cells loosely arranged, not storied; mesophyll nearly always distinctly differentiated into palisade and spongy parenchyma; druse crystals present in midrib area (usually in relatively large numbers) .... **(h) *C. spinosum***

The following brief notes on the diagnostic leaf characters of the various species supplement the key [for detailed descriptions of each species Tilney (1986) may be consulted].

(a) *C. inerme* (Figures 6, 7)

The usefulness of tanniniferous cells in the stem for the identification of certain southern African species or groups of species of *Canthium s.l.* has previously been mentioned (Tilney *et al.* 1988). The abundance and distribution pattern of these cells in the leaf is similar in

C. inerme and *C. suberosum*, being essentially of type-B. In discussing 'secretory cells, filled with brown contents' in *C. inerme* (= *Plectronia ventosa* L.), Solereder (1908) mentions their presence in the mesophyll 'where they form a middle layer in the leaf and also occur as dilated cells in the palisade-tissue', a pattern that corresponds with that of the specimens examined in the present study (Figure 7). *C. inerme* and *C. suberosum* were the only species studied that are completely glabrous. It would, therefore, seem that these two species are closely related. Despite the similarities in the distribution of type-B tanniniferous cells, the various anatomical features given in the key, especially in combination, can be used very successfully to separate them. The petioles of both species are virtually identical (Figure 6). The best, though not altogether satisfactory, criteria for their distinction are the number of vessels per radial tier, which are fewer in *C. inerme*, and the cuticle thickness which tends to be less than (although it can be similar to) that of *C. suberosum*. Petiolar features were, therefore, not used to distinguish between the two species in the key.

(b) *C. suberosum* (Figures 8–10)

Leaf anatomical features that point to a possible close relationship between *C. suberosum* and *C. inerme* and the diagnostic characters are mentioned in the discussion of *C. inerme*. With regard to the thickness of the leaf blade and midrib areas the minimum of the range observed for both parts is greater than that of the other taxa (Figure 4). The minimum value for the leaf blade of *C. suberosum* exceeds the maximum of the range of several species. However, the maximum leaf blade thickness recorded for *C. suberosum* is similar to that of *P. obovata* but the midrib thickness is exceeded by three species, all of which are members of the *Psydrax* group. When the ratio of the thickness of the midrib area to that

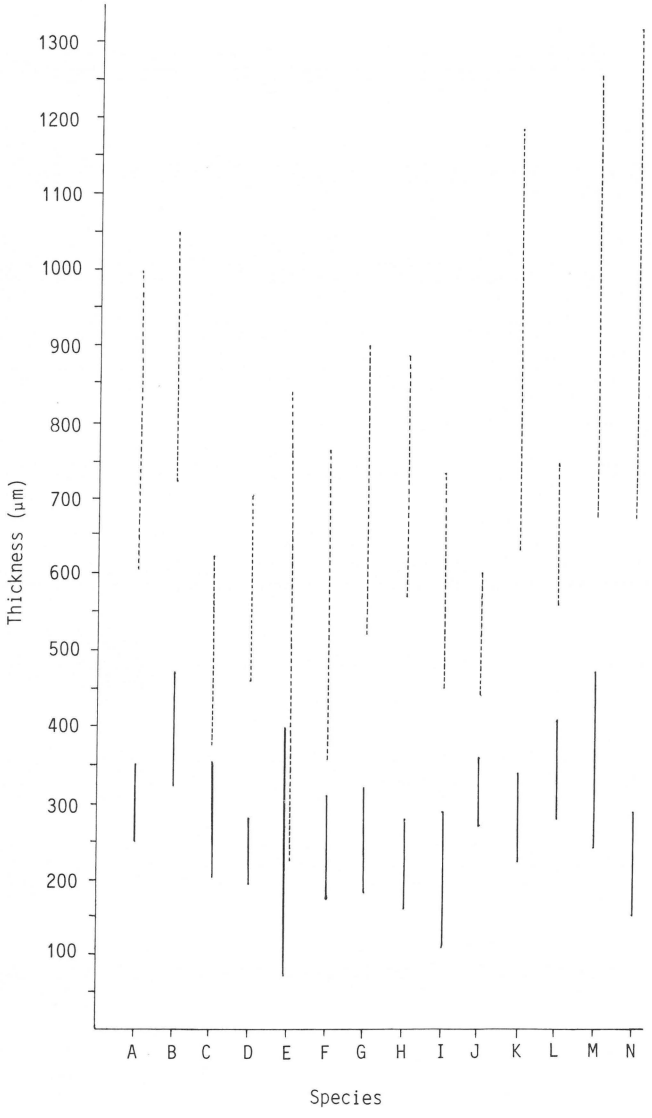


Figure 4 Graph of the range in thickness of the leaf blade and midrib area of some species of *Canthium s.l.* (A) *C. inerme*; (B) *C. suberosum*; (C) *C. pauciflorum*; (D) *C. setiflorum*; (E) *C. ciliatum*; (F) *C. vanwykii*; (G) *C. gilfillanii*; (H) *C. mundianum*; (I) *C. spinosum*; (J) *Psydrax fragrantissima*; (K) *P. livida*; (L) *P. locuples*; (M) *P. obovata*; (N) *Keetia gueinzii*. Leaf blade ——— , midrib area – – – .

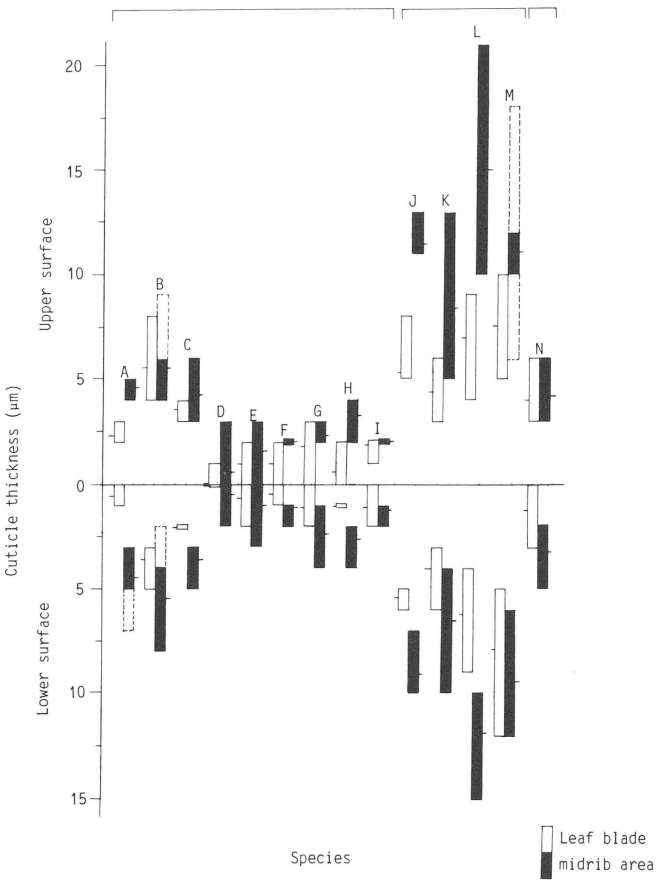
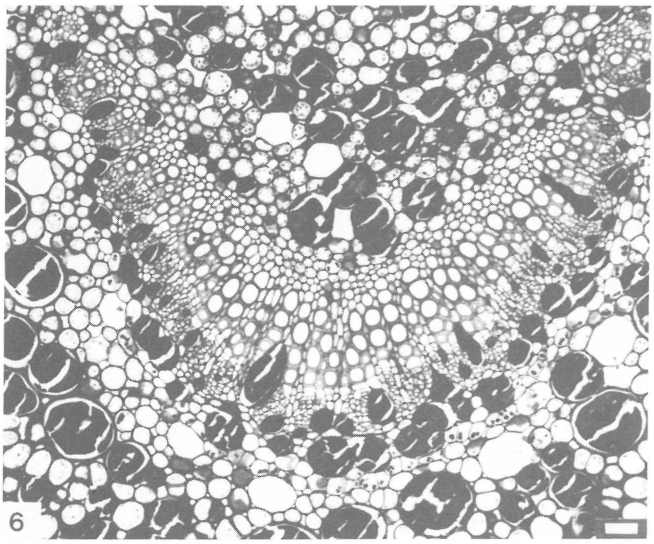
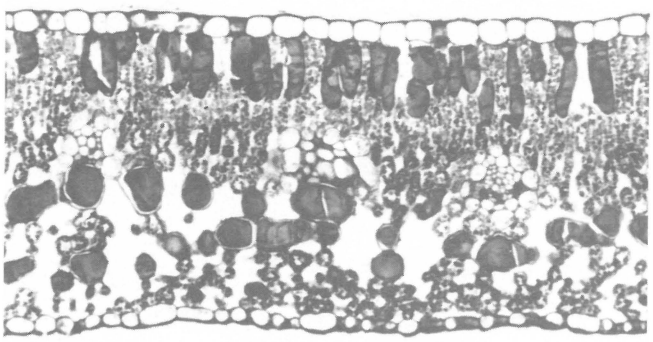


Figure 5 Histograms of the range in cuticle thickness of the leaf blade and midrib area of some species of *Canthium s.l.* (A) *C. inerme*; (B) *C. suberosum*; (C) *C. pauciflorum*; (D) *C. setiflorum*; (E) *C. ciliatum*; (F) *C. vanwykii*; (G) *C. gilfillanii*; (H) *C. mundianum*; (I) *C. spinosum*; (J) *Psydrax fragrantissima*; (K) *P. livida*; (L) *P. locuples*; (M) *P. obovata*; (N) *Keetia gueinzii*. The broken lines indicate single values that differ widely from the others. The average is indicated by a horizontal line.

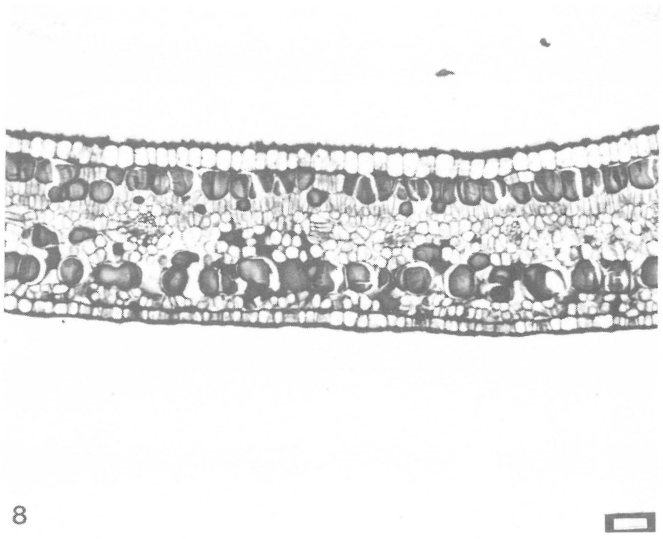
of the leaf blade is considered, it is generally found to be relatively low but not sufficiently so as to warrant its use for distinguishing between *C. inerme* and other taxa. As far as this ratio is concerned, *C. suberosum* shows a very small range of variation between the nine specimens examined.

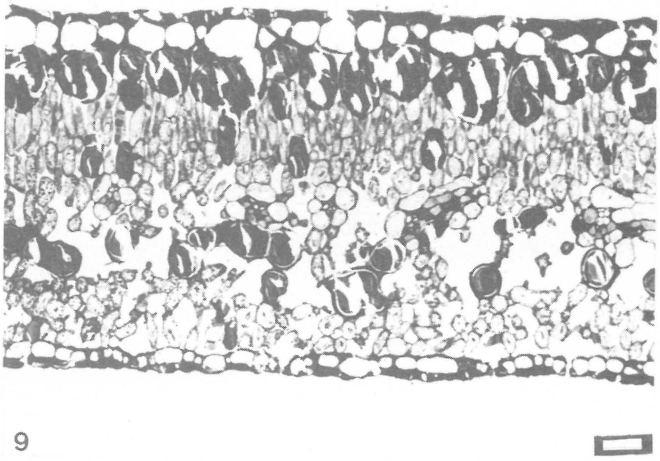
Comparison of the characters of the leaf blade that are regarded as being important for identifying this species, reveals no significant differences between immature and mature specimens of a single plant. Particularly noteworthy is the similar distribution pattern and abundance of the type-B tanniniferous cells in both age classes (Figures 8, 9). Although not as darkly stained as when fully developed, the cracked appearance of these cells is clearly evident in the immature stage. Minor differences that are apparent in the young state include the more compact spongy parenchyma and epidermal cells tending to be anticlinally elongated (Figures 8, 9). Although the

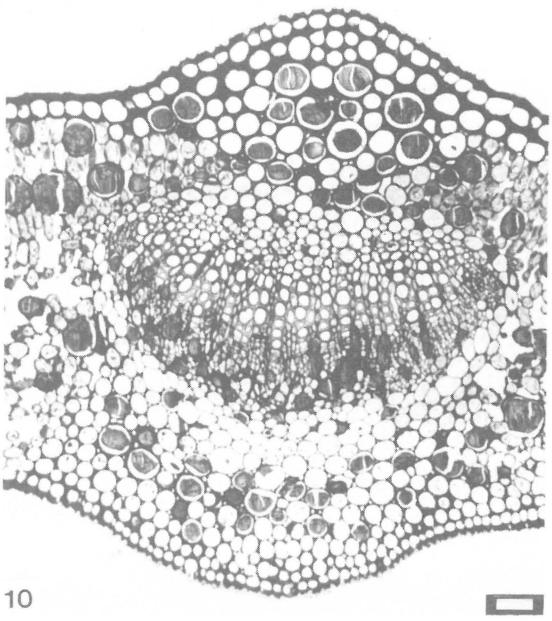




Figures 6 & 7 *Canthium inerme*. **6**. The central part of the petiole showing the relatively small number of vessels comprising each radial tier and type-B tanniniferous cells. *Abbott 889*. **7**. Part of the leaf blade showing the distribution pattern of the type-B tanniniferous cells. *Kok 732*. Scale bars = 50 μm.







Figures 8–10 *Canthium suberosum*. **8.** Part of the leaf blade of an immature leaf of showing the distribution pattern of the type-B tanniniferous cells. *Tilney 141*. **9**. Part of the leaf blade of a mature leaf showing the distribution pattern of the type-B tanniniferous cells. *Tilney 141*. **10**. Midrib area of the immature leaf showing the distribution pattern of the type-B tanniniferous cells. *Tilney 141*. Scale bars = 50 μm.

type-B tanniniferous cells are present in the midrib area, they are not quite as numerous in the immature material, particularly in the phloem, as in the fully developed leaf (Figure 10). It would seem from the limited study of this species and also of *C. gilfillanii* [see (g)] that the stage of development of a leaf is not crucial as far as the diagnostic characters are concerned and that it is possible for accurate identifications to be made irrespective of the age.

In view of the significance of the type-B tanniniferous cells for diagnostic purposes in the southern African species of *Canthium s.l.*, comparisons of these cells were made in fresh and rehydrated material. These cells appear essentially the same irrespective of the treatment. This further enhances their taxonomic usefulness.

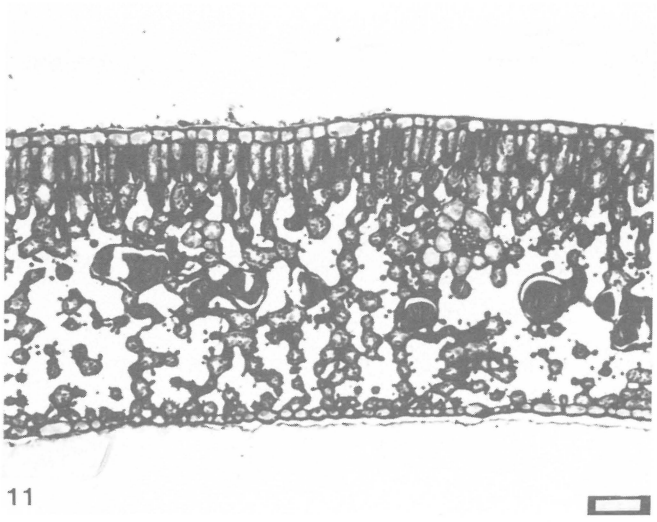
(c) *C. pauciflorum* (Figures 11, 12)

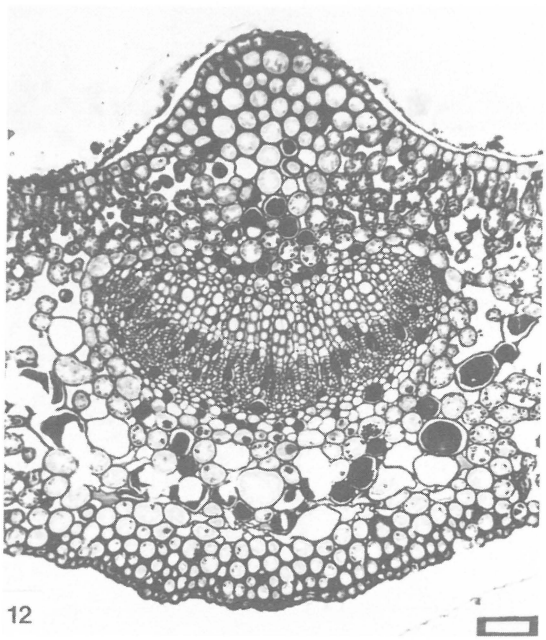
The most distinctive feature of this species appears to be the distribution pattern of the type-B tanniniferous cells in the leaf blade (Figure 11). These cells are confined to one or more essentially continuous layers in the spongy parenchyma tissue in the middle of the mesophyll. Another diagnostic character is the shape of the midrib area which, although conspicuously raised above, is broad and only slightly raised below (Figure 12). This is reflected in the measurements of the leaf blade and midrib area where it is found that there is little difference between the maximum of the range for the leaf blade and the minimum of the range for the midrib area (Figure 4). When the ratios of the thickness of these areas are studied, it can be seen that relatively little variation is encountered.

(d) *C. setiflorum* (Figures 13–15)

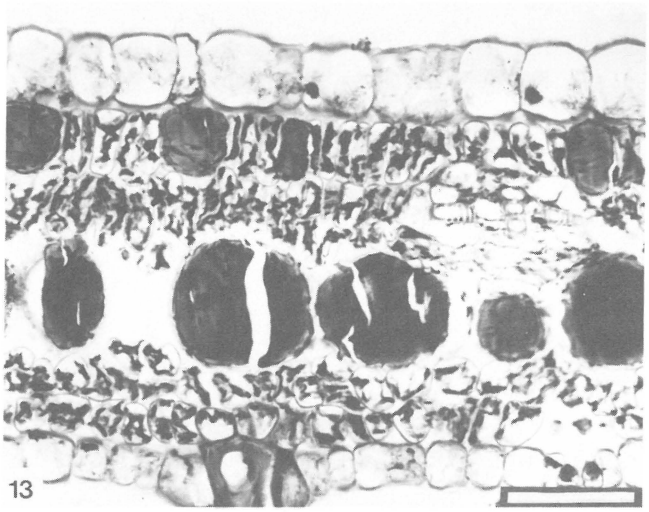
The distinct tanniniferous hairs of the leaf and stem are mentioned by Tilney *et al.* (1988). In addition, the distribution pattern of the tanniniferous cells (type-B) in the leaf blade is characteristic of the species (Figure 13). These cells are nearly always markedly dilated and therefore very conspicuous, making this a very useful diagnostic character. *C. setiflorum* resembles *C. gilfillanii* and *C. mundianum* and also the *Psydrax* group in that the spongy parenchyma of the leaf blade is compact and more or less storied (Figure 14). However, in *C. setiflorum* the differentiation of the mesophyll into palisade and spongy parenchyma tends to be a little more distinct and the mere presence of type-B tanniniferous cells would preclude any confusion. Unlike nearly all other members of *Canthium s.str.*, *C. setiflorum* has leaf blade epidermal cells of which the inner as well as outer periclinal walls tend to be heavily cutinized (Figure 13). Similar cutinized walls occur in *C. suberosum*. However, in addition to the diagnostic characters for *C. setiflorum* mentioned above, it has a very thin cuticle unlike that of *C. suberosum* which is fairly well developed.

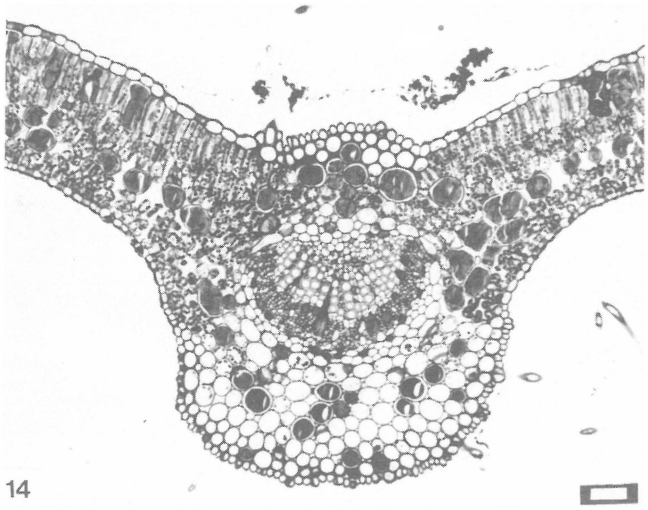
The tanniniferous cells (type-B) in the midrib area are present in larger numbers towards the upper epidermis than immediately above the xylem (Figure 14). This is in sharp contrast to the pattern observed in most other species where the type-B cells tend to be concentrated near the xylem. In most specimens of *C. setiflorum* the type-B tanniniferous cells in the midrib area stain (Periodic Acid-Schiff reaction and counterstained with toluidine blue) a different colour (blue rather than brown) and have relatively few 'cracks' compared with those in the leaf blade; some, especially towards the centre of the midrib area, are essentially without 'cracks' therefore appearing more like the type-A variety. However, in few specimens, the type-B tanniniferous cells have a similar appearance in the midrib area and leaf blade. It therefore seems possible that the type-B tanniniferous cells may initially develop fully in the leaf

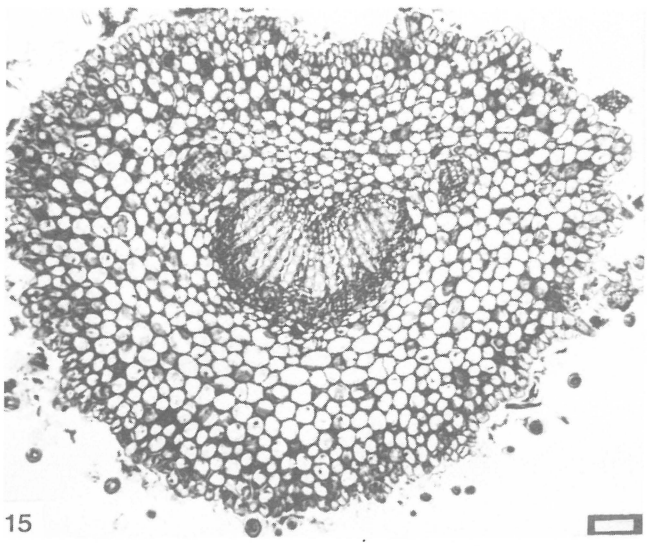




Figures 11 & 12 *Canthium pauciflorum.* **11.** Part of the leaf blade of *C. pauciflorum* showing the structure of the mesophyll and the distinctive distribution pattern of the type-B tanniniferous cells. *Tilney 188.* **12.** Midrib area of the leaf of showing the distinctive shape of the midrib and distribution pattern of the type-B tanniniferous cells. *Tilney 188.* Scale bars = 50 μm.

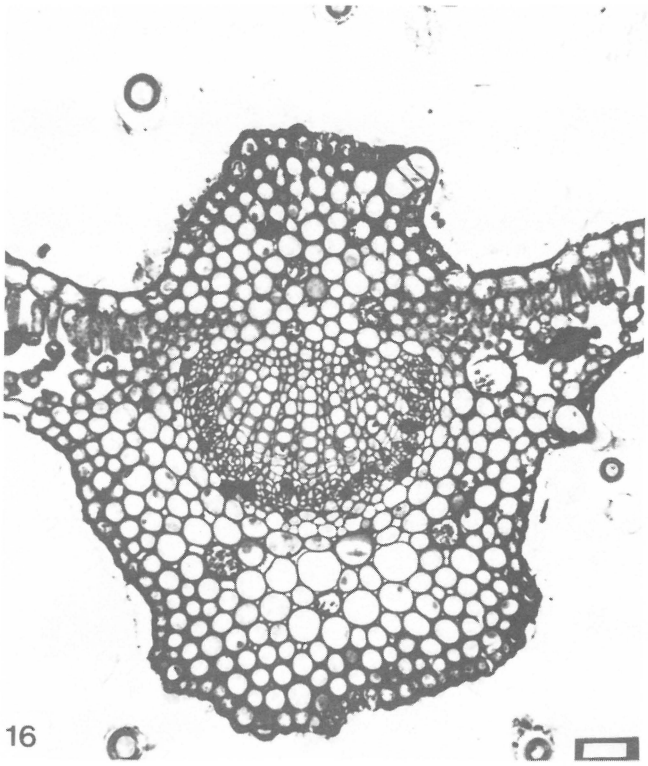


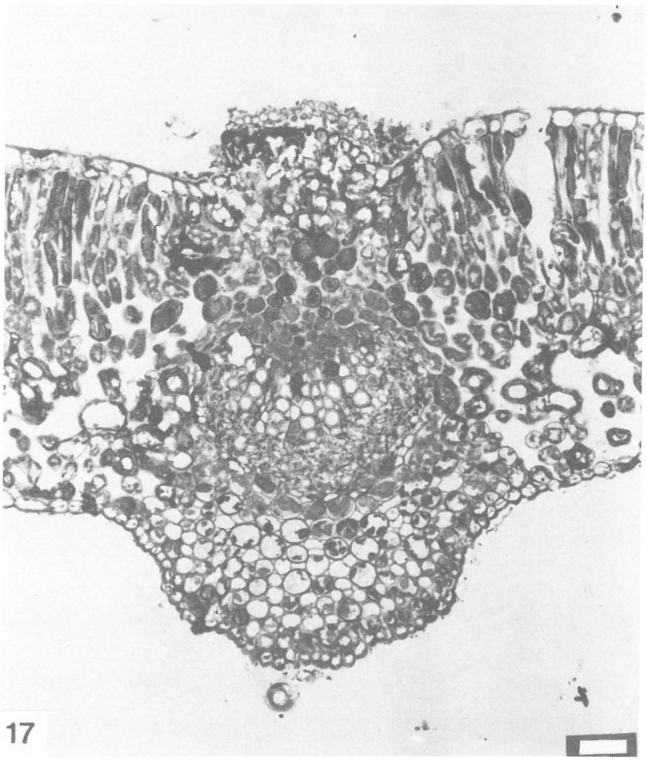




Figures 13–15 *Canthium setiflorum*. **13.** Part of the leaf blade of *C. setiflorum* showing the dilated appearance and characteristic distribution pattern of type-B tanniniferous cells. *Van Rooyen 1181*. **14.** Midrib area of the leaf (material from Ethiopia) showing the distribution pattern of type-B tanniniferous cells. *Puff 820909-1/1*. **15**. Petiole showing the concentration of type-B tanniniferous cells towards the outer regions of the mesophyll. *Van der Schijff 3562*. Scale bars = 50 μm.

blade and then progressively throughout the midrib area possibly by further deposition of tanniniferous substances or chemical change, to transform the type A-like cells into the typical type-B cells. A similar situation was observed in *C. suberosum* which lends support to



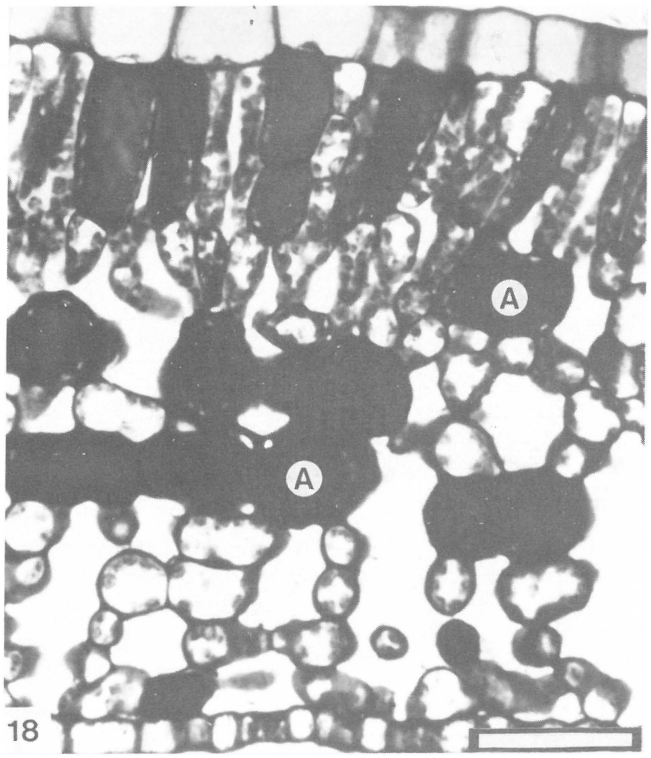


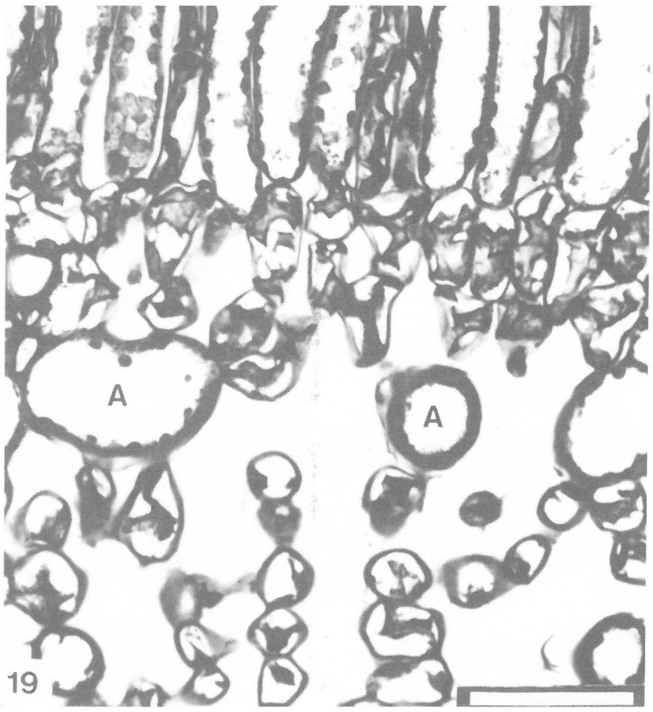
Figures 16 & 17 *Canthium ciliatum*. **16.** Shape of the midrib area of the leaf. Kok 730. **17**. Midrib area of the leaf showing the shape and large number of tanniniferous cells. *Van Wyk 3299*. Scale bars = 50 μm.

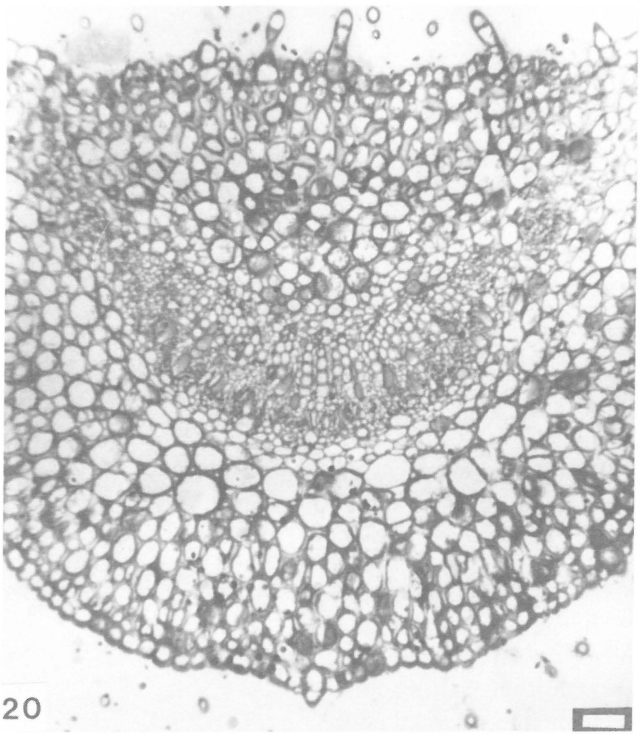
this suggestion. The immature leaves of *C. suberosum* have tanniniferous cells in the midrib area that are not as darkly stained or as 'cracked' as those in the leaf blade, although in the midrib area of mature leaves from the same plant (Figure 10) these cells appear essentially the same as in the leaf blade.

Measurements of the thickness of the midrib area and leaf blade, as well as the ratios of the thickness of the midrib area to the leaf blade, are intermediate between those encountered in other species (Figure 4), and these aspects do not seem to be of value for identification purposes.

The type-B tanniniferous cells in the petiole appear to be distributed in the mesophyll such that the greatest







Figures 18–20 *Canthium vanwykii*. **18.** Part of the leaf blade showing the appearance of the tanniniferous cells (A) in fresh material. *Tilney 152*. **19**. Part of the leaf blade showing the appearance of the tanniniferous cells (A) in rehydrated material. *Tilney 152*. **20**. Petiole showing the relatively large number of radial tiers of vessels. *Van Wyk 4189*. Scale bars = 50 μm.

concentration lies towards the outer regions rather than close to the vascular bundle as it occurs in many of the other species (Figure 15).

Leaf material of specimens of *C. setiflorum* obtained from Inhaca and Ethiopia was also studied. As far as the leaf blade, midrib area and petiole are concerned, the characters discussed above for the southern African specimens were found to be similar except that the inner periclinal cell walls of the epidermal cells of the leaf blade appeared less cutinized. The distribution pattern of the type-B tanniniferous cells in the leaf blade, a character which is of considerable taxonomic value in this species, was found to be similar to that of the southern African specimens, as was that of the midrib area.

(e) *C. ciliatum* (Figures 16, 17)

This species shows the most variation of all the taxa studied. Wide variation was encountered with the shape of the midrib area (Figures 16, 17), the thickness of the leaf blade and midrib area (Figure 4) and also the ratio of the thickness of these areas. In addition, the type-A tanniniferous cells (when present) in the various organ parts studied varied. Although the petioles of certain specimens of this species have the least number of radial tiers of vessels as well as vessels per tier, the range observed was so wide that the maximum values encountered exceeded those of certain other species.

The leaf blade varied in thickness from 70–400 μm, but most specimens of *C. ciliatum* had the thinnest leaves of all the southern African species. Furthermore, in all but one of the large number of specimens examined, the cuticle was found to be remarkably uniform in thickness, being relatively thin (Figure 5E) in all the parts studied. Hairs associated with the different organ parts have a similar basic structure but vary much in density in the various specimens. Despite the variation encountered, it appears possible to identify most specimens correctly by using the key.

(f) *C. vanwykii* (Figures 18–20)

Although this species is morphologically distinct, diagnostic anatomical characters proved difficult to find. In several respects it resembles certain specimens of *C. ciliatum*, a species in which a relatively large amount of variation is observed. The superficial vegetative resemblance between these two species as well as a selection of differentiating morphological characters are mentioned by Tilney & Kok (1987). Some of the anatomical similarities, indicative of a possible relationship between these taxa, are the following:

a. The mesophyll of the leaf blade is generally similar (Figure 19). In some specimens of *C. vanwykii* (Figure 18) the taniniferous cells present in the leaf blade have a distribution patern that resembles that of some specimens of *C. ciliatum*. In these specimens the tannin cell distribution pattern in the midrib area is also similar.
b. In both species some of the type-A tanniniferous cells are distinctive, appearing to be vacuolated.
c. Both species have a very thin cuticle on both surfaces of the leaf blade and midrib area.
d. An equal number (*c.* 10–24) of radial tiers of vessels in the midrib area and petiole (Figure 20) was observed, although this number is not confined to these species.

Some of the anatomical differences, which in combination may assist in distinguishing these species, are supplied in the key.

The tanniniferous cells in *C. vanwykii* may be particularly difficult to identify especially in material that has not rehydrated satisfactorily. A comparison between fresh and rehydrated material from the same plant is shown in Figures 18, 19. In the rehydrated specimen (Figure 19) the tanniniferous cells appear as dilated cells with the tanniniferous deposits concentrated around the periphery of the cells and are therefore, at least initially, more difficult to detect. It was also noted that in the midrib area of specimens in which the type-B tanniniferous cells are absent, the type-A tanniniferous cells tend to be numerous and include some cells that are brown but do not have the typical 'cracked' appearance of cells regarded as being type-B. It therefore seems that further studies of the tanniniferous substances may reveal these to be of a distinctive type in *C. vanwykii*.

(g) *C. gilfillanii* and *C. mundianum* (Figures 21–23)

Of all the taxa under consideration, *C. gilfillanii* is the most densely pubescent. Anatomically it is essentially only the degree of pubescence that distinguishes the leaf of this species from *C. mundianum*. On the petiole the position of the hairs also appears to differ, these being more or less evenly distributed in *C. gilfillanii*, but concentrated on the upper side in *C. mundianum*. In many of the other species, variation in the extent of pubescence was encountered and it seems that a species distinction, based largely on pubescence, is highly questionable. This is particularly so in view of the large number of anatomical similarities noted between these two species. Jeppe (1964, 1969) made a distinction between the two species according to the height and symmetry of the plant, as well as leaf characters including hairiness. Anatomically, however, some of the main similarities include the following:

a. Side veins prominently raised and conspicuous.
b. Mesophyll rarely distinctly differentiated into palisade and spongy parenchyma; spongy parenchyma cells nearly always compact and storied (Figure 21). This is unlike that of other members of *Canthium s.str.*
c. Type-B tanniniferous cells are usually absent from the leaf blade except in the larger side veins or occasionally in short rows in the mesophyll.
d. In the midrib area type-B tanniniferous cells are usually present in the phloem and in the mesophyll above and below the vascular bundle in varying numbers (Figure 22).

e. Petiole structure, apart from pubescence, is similar (Figure 23).

It is clear that the anatomical observations support the treatment of these taxa as a single species (e.g. Coates Palgrave 1977).

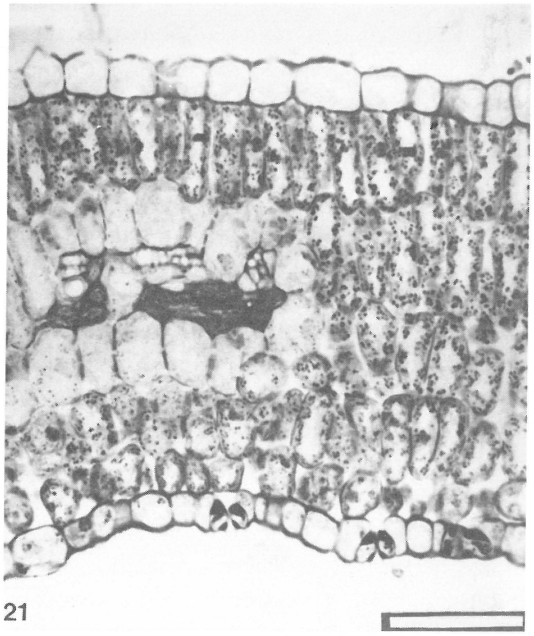
Several specimens of *C. gilfillanii* and *C. mundianum* were selected from within the Johannesburg area (as well as from other localities) to investigate the nature and extent of any local variation that may exist (Table 1). Intraspecific variation was minimal — details are given in Tilney (1986). Therefore, in these taxa, it does not appear that more variation is likely to be encountered between widely separated specimens than between those growing in fairly close proximity.

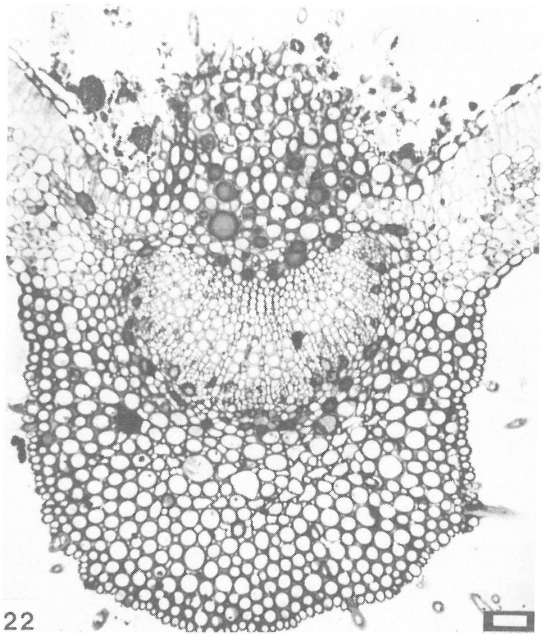
Specimens of *C. gilfillanii* as well as of *C. suberosum* were used to compare the anatomy of immature and mature leaves. In both species, even at a very early stage of development, type-B tanniniferous cells are clearly visible. In the midrib area of *C. gilfillanii* these cells are one of the most striking features of the immature leaf and their distribution pattern is similar to that of the mature leaf (Figure 22). The petiole of the immature leaf possesses conspicuous tanniniferous cells with the same distribution pattern as was found in other specimens from different plants.

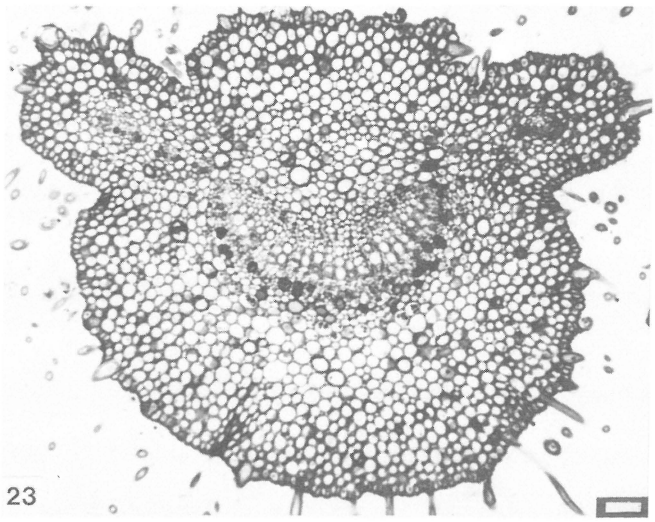
A comparison of fresh and rehydrated material was made with special reference to the appearance of the tanniniferous cells. In rehydrated material type-B tanniniferous cells are readily discernible but type-A may be difficult to detect.

(h) *C. spinosum* (Figures 24, 25)

The leaf of *C. spinosum* is essentially devoid of, or poor in, tanniniferous cells (Figure 24) as in the stem (Tilney

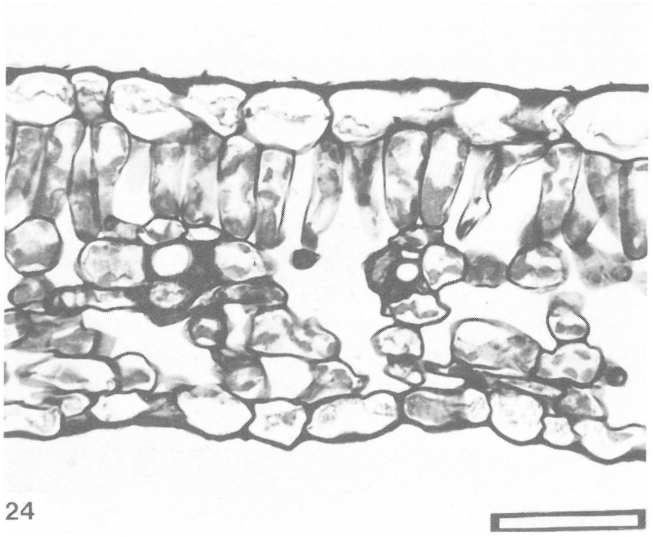


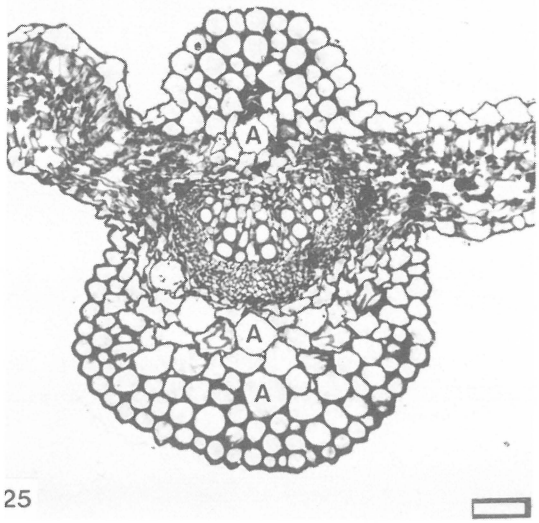




Figures 21–23 *Canthium gilfillani*. **21.** Part of the leaf blade showing the mesophyll indistinctly differentiated into palisade and spongy parenchyma. *Tilney 7*. **22.** Midrib area of the leaf showing the shape as well as the extensive collenchyma and distribution pattern of the tanniniferous cells. *Tilney 138*. **23.** Petiole showing the distribution pattern of the tanniniferous cells and the abundance of the hairs. *Tilney 7*. Scale bars = 50 μm.

et al. 1988). In the general lack of tanniniferous cells, this species resembles *C. gilfillanii* and *C. mundianum* as well as members of the *Psydrax* group and occasional specimens of *C. ciliatum*. The shape of the midrib area, being conspicuously raised above and very conspicuously projecting below (Figure 25), resembles that of *C. gilfillanii* (Figure 22) and *C. mundianum*. However, when the measurements, particularly of the midrib area, are considered it is seen that it tends to be thinner in *C. spinosum* (Figure 4). In all three species the ratio of the thickness of the midrib area to that of the leaf blade gives a wide range of values. Druse crystals (crystalliferous idioblasts referred to as 'ghost cells' if the crystals are dissolved during staining) are usually a conspicuous feature of the midrib area (Figure 25).





Figures 24 & 25 *Canthium spinosum*. **24.** Part of the leaf blade showing the structure of the mesophyll and the small number of tanniniferous cells. *Tilney 120*. **25.** Midrib area of the leaf showing the shape, 'ghost cells' (crystalliferous idioblasts, druse crystals dissolved during staining) (A) and the small number of tanniniferous cells. *Theron 1584*. Scale bars = 50 μm.

2. *Psydrax*

Leaf anatomical key to the species of *Psydrax* (excluding petiole)

1a Leaves amphistomatic.
- 2a Hairs absent in midrib area; midrib area conspicuously raised above but less conspicuously below; vessels of main vascular bundle of midrib in 14–18 radial tiers .... **(a) *P. fragrantissima***
- 2b Hairs present in midrib area; midrib area fairly broadly and conspicuously raised above and below; vessels of main vascular bundle of midrib in about 18–55 radial tiers ..... **(b) *P. livida***

1b Leaves hypostomatic.
- 3a Type-B tanniniferous cells absent from the secondary veins and mesophyll of the leaf blade; midrib area conspicuously raised above and below; outer periclinal cell walls of epidermal cells of midrib area slightly cutinized; palisade cells adjacent to epidermis nearly always elongated and narrow, essentially 2-layered .... **(c) *P. locuples***
- 3b Type-B tanniniferous cells often associated with secondary veins or in tangential rows in the mesophyll of the leaf blade; midrib area broadly and usually conspicuously raised above and below; outer periclinal cell walls of epidermal cells of midrib area considerably cutinized; palisade cells adjacent to epidermis nearly always short and broad, 2–5-layered ..... **(d) *C. obovata***

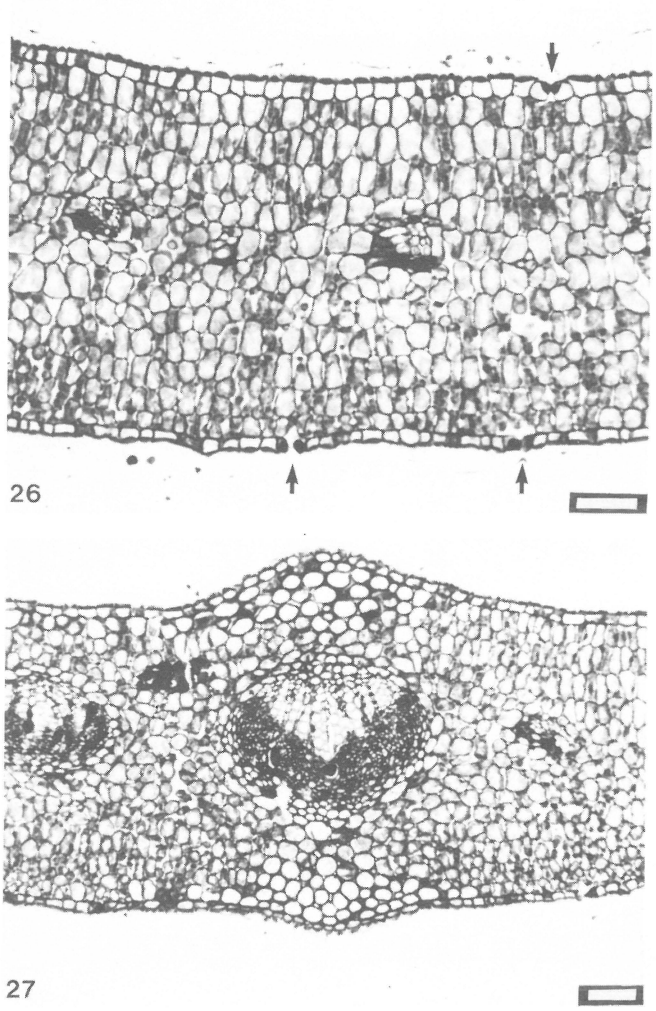
The following brief notes on the diagnostic leaf characters of the various species supplement the key [for detailed descriptions of each species Tilney (1986) may be consulted].

(a) *P. fragrantissima* (Figures 26, 27)

The leaves of all specimens of *P. fragrantissima* and *P. livida* examined were amphistomatic (Figures 26, 27). This character appears to be useful in distinguishing these species from the other *Psydrax* taxa. Table 3 summarizes differences between the two amphistomatic species.

Some uncertainty exists about the taxonomic status of *P. fragrantissima* and *P. locuples* (= *Plectronia locuples*). Codd (1960/61) felt that these species were so similar that they should be regarded as conspecific. Coates Palgrave (1977) presumably did not recognize *Psydrax fragrantissima* as a separate species since no mention of it is made in his work. Anatomically these taxa can be distinguished from each other as shown Table 4.

Cavaco (1972) considers *P. fragrantissima* (= *Canthium fragrantissimum*) and *P. obovata* (= *Canthium obovatum*) to be distinct since he mentions that certain species of *Canthium* from Madagascar have affinities with each of these African species. From her morphological studies, Bridson (1985) found these species to be 'very close'. The main differences are that *P. fragrantissima* has smaller leaves and, in particular, smaller flowers. She recommends that their present status be



Figures 26 & 27 *Psydrax fragrantissima*. **26.** Part of the leaf blade showing stomata (arrowed) on both surfaces and the relatively large number of palisade layers. *Gerstner 4811*. **27.** Midrib area of the leaf showing the distinctive shape, absence of hairs and the distribution pattern of the type-B tanniniferous cells. *Edwards 2962*. Scale bars = 50 μm.

maintained although future studies, including those of mature fruits, may suggest the recognition of *P. fragrantissima* as a variety of *P. obovata*. This anatomical study has indicated some similarity between these two species especially with regard to the tanniniferous cells. Some of the anatomical differences between these closely related species are given in Table 5. The relatively few differences observed support a close relationship between these two species and their leaf anatomy is very similar in all other respects.

(b) *P. livida* (Figures 28 & 29)

Psydrax livida can be readily separated from *P. locuples* and nearly always from *P. obovata* due to the distribution of the stomata (Figure 28). A further distinguishing character between *P. livida* and these hypostomatic species is the distribution of the type-B tanniniferous cells (Figures 1, 28). In *P. locuples*, unlike *P. livida* (Figure 29), these cells are absent from the mesophyll above and below the phloem and the vascular bundle of the midrib area (Figure 31). In *P. obovata* these cells may be absent in the leaf blade of the so-called group A members [see (d)] (Figure 33) as in *P. livida*, but are present in the group B members. However, in the midrib area of the group A members, those cells were not observed in the mesophyll above and below the vascular bundle, whereas in *P. livida* such cells are always present above and usually also below the vascular bundle (Figure 29). The occurrence of these cells is far more extensive in the petiole of *P. obovata* (Figures 1, 36, 37). Of the *Psydrax* group, *P. livida* tends to have the thinnest cuticle, particularly over the midrib area. Since it occurs in localities together with others in the *Psydrax* group, there appears to be no explanation for this in terms of the habitat.

The shape of the midrib area of *P. livida* differs from that of *P. fragrantissima* and *P. locuples* but may be fairly similar to that of *P. obovata* (Figure 35). As far as the

Table 3 Leaf anatomical differences between *P. fragrantissima* and *P. livida*

	P. fragrantissima	*P. livida*
Leaf blade	palisade 3–5-layered	palisade (1)2(3)-layered
Midrib area	conspicuously raised above but less conspicuously raised below (Figures 2J, 4J)	fairly broadly and conspicuously raised above and broadly and conspicuously raised below (Figures 2K, 4K)
	relatively thin (Figure 4J)	relatively thick (Figure 4K)
	hairs absent	hairs present
	epidermal cells generally not conspicuously papillate	epidermal cells generally conspicuously papillate
	type-B tanniniferous cells in phloem and in mesophyll below but only immediately adjacent to phloem; very rarely above vascular bundle	type-B tanniniferous cells in phloem and in mesphyll below but not confined to area immediately adjacent to phloem; nearly always above vascular bundle
	vessels in 14–18 radial tiers	vessels in 18–55 radial tiers
Petiole	vessels in ± 28 radial tiers	vessels in ± 55 radial tiers
	maximum number of vessels per tier 6–12	maximum number of vessels per tier 12–16

Table 4 Leaf anatomical differences between *P. fragrantissima* and *P. locuples*

	P. fragrantissima	*P. locuples*
Leaf blade	amphistomatic	hypostomatic
Midrib area	conspicuously raised above, but less conspicuously raised below (Figures 1, 2, 27)	conspicuously raised above and below (Figures 1, 2, 31)
	relatively thin	relatively thick
Petiole	type-B tanniniferous cells relatively widely distributed, and in large numbers in the phloem or its vicinity	type-B tanniniferous cells relatively limited in distrition, and in small numbers in the phloem or its vicinity

Table 5 Leaf anatomical differences between *P. fragrantissima* and *P. obovata*

	P. fragrantissima	*P. obovata*
Leaf blade	amphistomatic	hypostomatic
Midrib area	conspicuously raised above but less conspicuously raised below (Figure 4J)	broadly and usually conspicuously raised above and below (Figure 4M)
	relatively thin	relatively thick
Petiole	relatively small (less than 1 mm thick)	relatively large (1 mm or more thick)

Table 6 Leaf anatomical differences between *P. locuples* and *P. obovata*

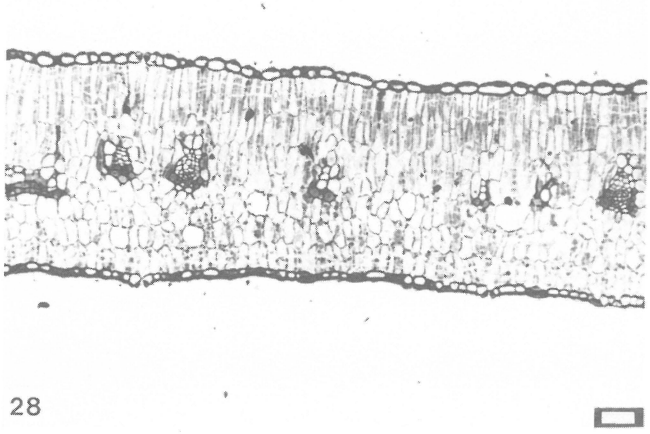
	P. locuples	*P. obovata*
Leaf blade	palisade cells tend to be elongate and narrow	palisade cells tend to be short and wide
Midrib area	thickness varies between about 560–740 μm	thickness varies between about 670–990(–1260) μm
	narrowly projecting above and below (Figures 2K, 4L)	broadly projecting above and below (Figures 2M,N, 4M)
	outer periclinal cell walls of epidermal cells slightly cutinized	outer periclinal cell walls of epidermal cells considerably cutinized
Petiole	cuticle about 10–12 μm thick	cuticle about 5–6 μm thick
	type-B tanniniferous cells absent from the mesophyll above the vascular bundle and from the xylem	type B tanniniferous cells present in the mesophyll above the vascular bundle and in the xylem

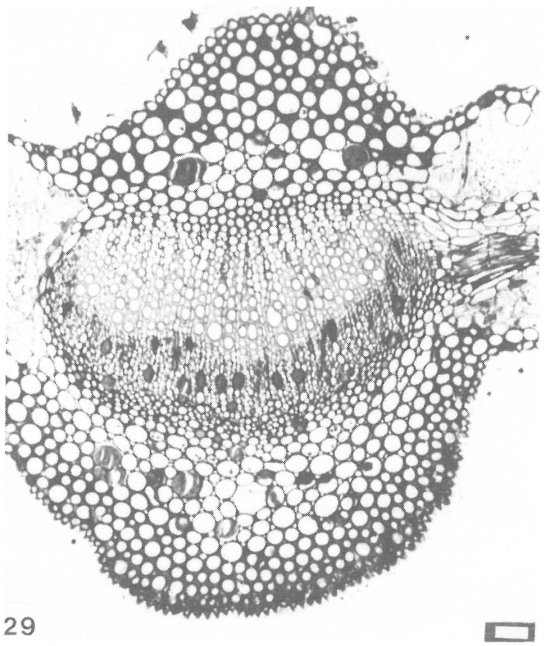
thickness of the leaf blade is concerned, *P. livida* has a range that tends to be slightly thinner than that of the other members of the *Psydrax* group (Figure 4). The thickness of the midrib area in relation to that of the leaf blade is, however, considerably greater than in the other members of the *Psydrax* group. The thickness of the midrib area, nevertheless, is very similar to that of *P. obovata* (Figure 2). Another feature of the midrib area of *P. livida* is the general presence of conspicuously papillate epidermal cells (Figure 29), a character not usually as well developed in the other species. The midrib area and petiole of *P. livida* appear to have the most extensive vessel system of the *Psydrax* group, as well as of the *Canthium s.str.* group. *K. gueinzii*, with the xylem arranged in a distinctive invaginated arc, has the largest number of radial tiers of vessels, but the number of vessels per tier is considerably less. This is a useful character for distinguishing *P. livida* from the other species.

(c) *P. locuples* (Figures 30–32)

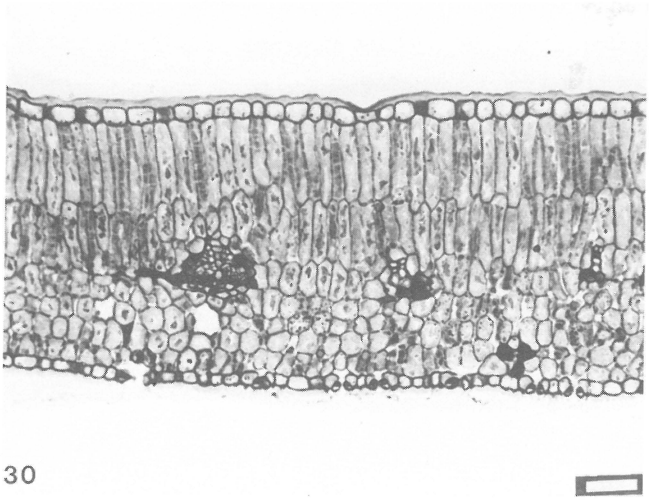
The leaves of this species contains the least number of tanniniferous cells of the *Psydrax* group and probably of all the taxa under study, especially as far as to the type-B tanniniferous cells are concerned (Figures 30, 31). However, the type-A tanniniferous cells, which gener-

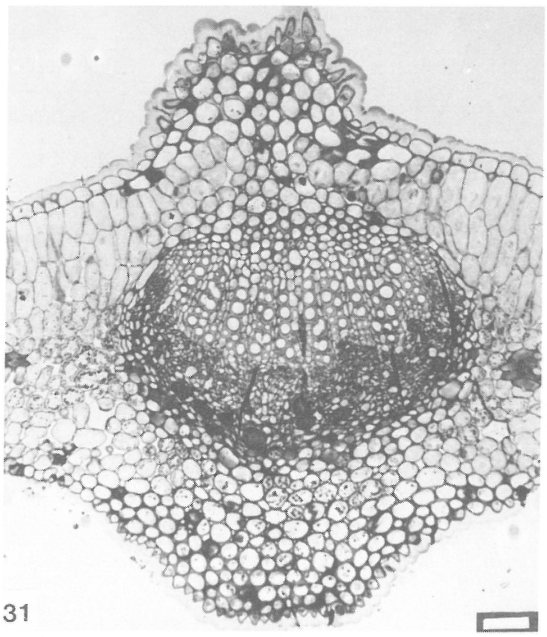
ally tend to be a relatively conspicuous feature of the *Psydrax* group, particularly of the leaf blade, are prominent also in the midrib area and petiole (Figure 32). The shape of the midrib area is one of the most useful characters for identification purposes, being conspicuously raised on both sides (Figure 2, 31). Its maximum thickness is also considerably less than that of *P. livida and P. obovata* (Figure 4). Ways of distinguishing *P. locuples* from *P. fragrantissima* and *P. livida* are mentioned in the respective discussions of these species [see (a) and (b)]. The morphological similarities between *P. locuples* and *P. obovata* have been pointed out by authors such as Codd (1960/61), Palmer & Pitman

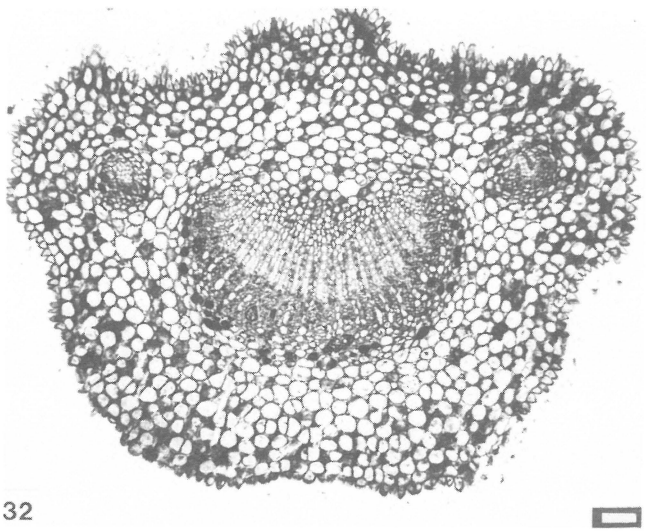




Figures 28 & 29 *Psydrax livida*. **28.** Part of the leaf blade showing the presence of stomata on both surfaces, the structure of the mesophyll and several 'ghost cells' (crystalliferous idioblasts). *Tilney 34*. **29.** Midrib area of the leaf showing the shape, conspicuously papillate epidermal cells, extensive xylem and the distribution pattern of type-B tanniniferous cells. *Kok 734*. Scale bars = 50 μm.





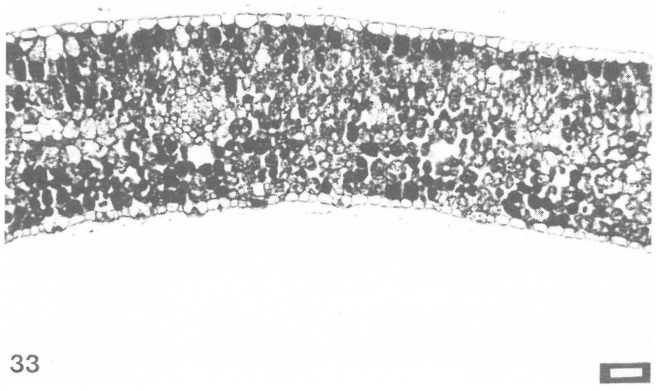


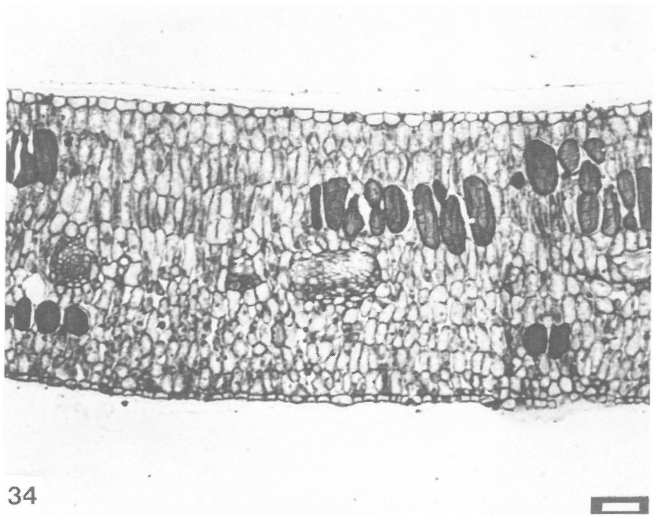
Figures 30–32 *Psydrax locuples*. **30.** Part of the leaf blade showing the structure of the mesophyll. *Edwards 1649*. **31.** Midrib area of the leaf showing the distinctively shaped thick cuticle and type-A tanniniferous cells. *Moll 4367*. **32.** Petiole showing the distribution pattern of the tanniniferous cells and the papillate epidermal cells. *Moll 4367*. Scale bars = 50 μm.

(1972) and Coates Palgrave (1977) who have distinguished between these species only on floral and fruit characters. Anatomically, however, it is usually possible to distinguish readily between them. Some of these anatomical differences are summarized in Table 6.

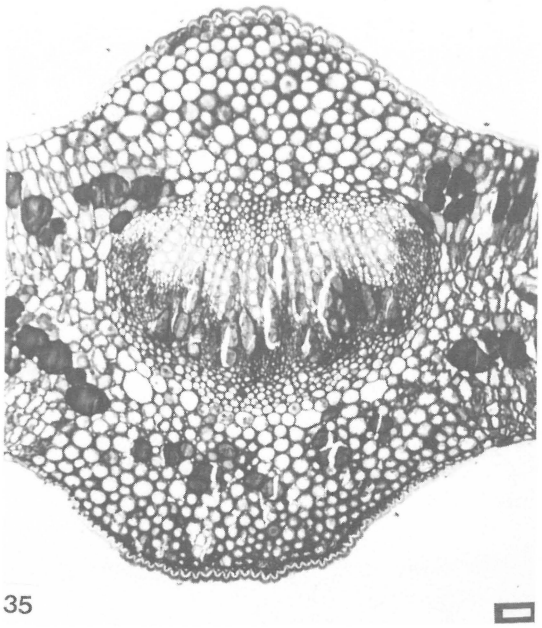
(d) *P. obovata* (Figures 33–36)

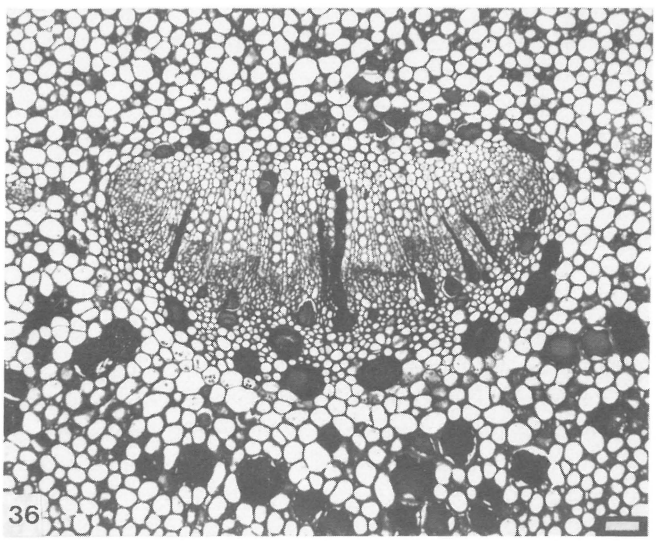
In most members of the *Psydrax* group, the distribution pattern of the type-B tanniniferous cells is useful for diagnostic purposes (Figure 1, 36). In *P. obovata* there appear to be two distinct patterns which are particularly evident in the leaves, suggesting the possible recognition of infraspecific categories (as proposed by Bridson 1985). In the one group, referred to as the A group, there are far fewer type-B tanniniferous cells than in the other so-called B group. The A group is characterized by the absence of these cells in the leaf blade (Figures 3, 33), but they are present in varying numbers in the phloem of the midrib area and occasionally may also occur in small numbers in the xylem and in the mesophyll above and immediately below the vascular bundle. In this group many mesophyll cells are often found to contain tanniniferous deposits of an apparently different kind (stain brownish-blue with toluidine blue) as that observed in other species. In the B group, the type-B tanniniferous cells are present in the leaf blade arranged in rows of varying length (but usually relatively short) in the palisade and spongy parenchyma (Figure 34). Occasionally they may be absent from the spongy parenchyma or occur as single cells. As in *C. pauciflorum*, they are not positioned adjacent to the upper epidermis (Figure 3). In the midrib area they are usually present in large numbers in the phloem and in relatively large numbers in the xylem (Figure 35). They may also be situated in the mesophyll above and below (adjacent





Figures 33 & 34 *Psydrax obovata*. **33.** Part of the leaf blade (group A) showing the absence of type-B and the presence of type-A tanniniferous cells. *Kok 729*. **34**. Part of the leaf blade (group B) showing the distribution pattern of type-B tanniniferous cells. *Tilney 131*. Scale bars = 50 μm.





Figures 35 & 36 *Psydrax obovata (group B)*. **35**. Midrib area of the leaf blade showing the shape and the distribution pattern of the tanniniferous cells. *Van Wyk A166*. **36**. The central part of the petiole showing the distribution pattern of the tanniniferous cells. *Tilney 131*. Scale bars = 50 μm.

to the phloem or further below) the vascular bundle. This B group represents the only instance in the *Psydrax* group where type-B tanniniferous cells occur in the leaf blade. The anatomical groups A and B correspond with the subspecies *elliptica* and *obovata* proposed by Bridson (1985) on the basis of various macromorphological characters.

Of the 11 specimens of *P. obovata* studied, one was found to be exceptional in having amphistomatic leaves. No further exceptions were observed in any of the other species under study. The character of stomatal position therefore appears to be reasonably reliable. As this feature was used in the key based on anatomical characters of the leaf blade, the amphistomatic specimen of *P. obovata* could not be keyed out successfully. However, it would be obvious that it could not be *P. livida or P. fragrantissima*, since the hairs and the shape of the midrib area would differ.

Several similarities and differences between *P. obovata* and the other species of the *Psydrax* group are mentioned under the other species [see (a–c)].

The anatomy of the leaf blade and midrib area of the southern African specimens was compared with that of material from Inhaca. This latter material was found to be essentially similar as far as the main diagnostic characters are concerned, differing in that only a few type-B tanniniferous cells were observed in the leaf blade. However, the similarity is more marked towards the midrib area where these cells become more numerous. In addition, the spongy parenchyma appears to be somewhat less compact. This specimen therefore belongs to the B group (subspecies *obovata*) and its locality is in accordance with the distribution shown by Bridson (1985).

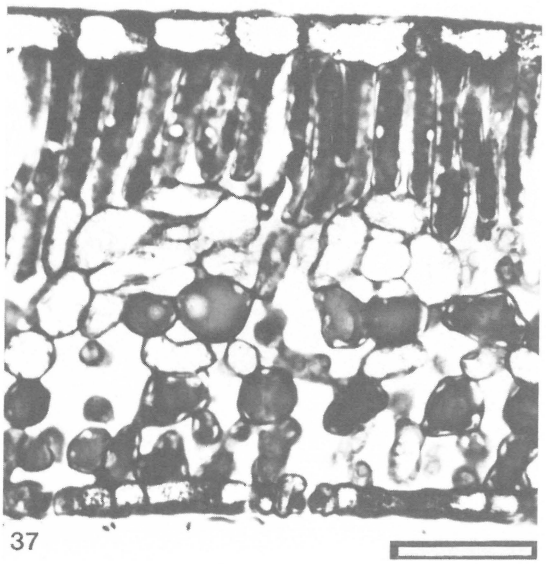
3. *Keetia* (only one southern African species, *K. gueinzii*) (Figures 37, 38)

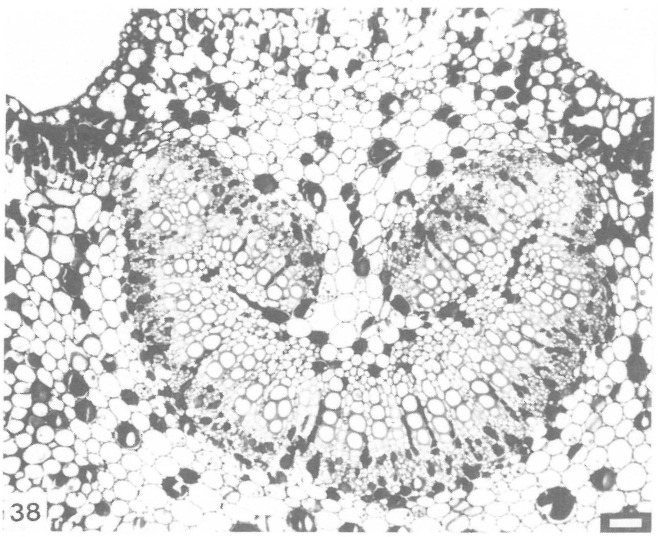
Morphologically this taxon is the most distinct of the southern African species of *Canthium s.l.* This is substantiated by the anatomy of the leaf in particular (midrib area and petiole) where the main vascular bundle, being an arc with invaginated ends, is unlike that of any of the other species (Figure 38). A further diagnostic feature is the hairs associated with the leaves and young stems (Tilney *et al.* 1988). The large number of these tanniniferous hairs and cells present (Figure 37) account for the brownish colour of the leaves and young stems, which is particularly conspicuous after drying. Another characteristic feature is the broad and conspicuously raised midrib area (Figure 2). When the thickness of the leaf blade is considered, it can be seen that the maximum thickness is similar to, or exceeded by, nearly all the other species (Figure 4). The midrib area is, however, relatively thick, attaining a maximum thickness greater than that of any of the other taxa. This is reflected in the values of the ratio of the relative thickness of the midrib area and leaf blade. The minimum value exceeds that of the other species as does the maximum of the range.

A leaf of a specimen from Ethiopia, tentatively identified as *K. gueinzii*, was examined anatomically. In general, the leaf blade resembles the southern African specimens, but the shape of the main vascular bundle was different. If the identification is correct and such a difference actually exists, it would be interesting to examine further specimens from different localities. These could be widely separated as this species is claimed to be the most widespread in Africa (Bullock 1932).

Conclusions

The present study clearly indicates significant leaf anatomical differences between nearly all the southern African taxa of *Canthium s.l.* On the basis of leaf





Figures 37 & 38 *Keetia gueinzii*. **37**. Part of the leaf blade showing the large number of type-A tanniniferous cells. *Kok 712*. **38**. Midrib area of the leaf showing the vascular bundle in a form of an arc with invaginated ends. *Kok 712*. Scale bars = 50 μm.

anatomy, as was the case with stem anatomy (Tilney *et al.* 1988), three groups were identified which were found to correspond with the three genera of *Canthium s.l.*, namely *Canthium s.str.*, *Psydrax* and *Keetia*, proposed by Bridson (pers. comm., 1985, 1986) following a morphological study of herbarium specimens. For example hairs, where present, are similar on leaves and young stems and the different hair types noted in the three groups enable successful placement of the various species in one of these segregate genera. As far as the other differentiating characters are concerned (Table 2), it can be seen that *Canthium s.str.* differs markedly in most respects from the *Psydrax* group but has some features in common with the *Keetia* group, such as mesophyll that is usually distinctly differentiated into palisade and spongy parenchyma.

Leaf anatomical characters are even more useful than stem anatomical features and are very reliable in distinguishing nearly all specimens of the 14 southern African species of *Canthium s.l.* Stems and leaves studied revealed striking similarities between *C. gilfillanii* and *C. mundianum*. It is essentially only on the basis of the degree of pubescence that these species can be distinguished from one another. Since several anatomical features were confined to these two species, they can readily be distinguished from the other taxa and this strongly suggests that they are conspecific.

The specimens of *P. obovata* showed anatomical differences indicating that two groups exist within this species. Differences in the distribution of the type-B tanniniferous cells were particularly apparent. These two anatomical groups generally correspond with the two subspecies proposed by Bridson (pers. comm., 1985).

This anatomical study also provides evidence indicating that *P. fragrantissima* and *P. locuples* are distinct from one another. For example, the leaves of the former species are amphistomatic, whereas those of the latter are hypostomatic. In this study several anatomical characters also point to relationships between certain southern African species. *C. inerme* and *C. suberosum*, for example, have several features in common, including an exceptionally large number of type-B tanniniferous cells which suggest that these species are closely related. As was observed in the stem, *C. ciliatum*, unlike the other species, showed wide variation in a number of features such as the shape of the midrib area. Leaf material of *C. setiflorum* and *P. obovata* obtained from localities outside southern Africa was essentially similar to the southern African specimens. This further substantiates the constancy of the diagnostic characters used.

Characters found to be particularly important for taxon recognition are as follows: the distribution pattern of the type-B tanniniferous cells, particularly in the mesophyll of the leaf, is diagnostic for many taxa (Figures 1, 2, 3); the structure of hairs which aids substantially with the separation of groups but is also useful for recognizing *C. setiflorum*; the density and position of hairs, especially in conjunction with other characters; cuticle thickness which is also significant for identifying groups and with the recognition of *C. suberosum*; the occurrence of stomata on one or both sides of the leaf which aids in distinguishing between certain species of the *Psydrax* group; aspects relating to the structure of the mesophyll of the leaf blade such as the degree of differentiation into palisade and spongy parenchyma, and the arrangement of the spongy parenchyma cells (compact/loose and storied/not storied) which assist in distinguishing between taxa at the generic level and also, in some cases, at the specific level; midrib areas which are a characteristic shape for certain species of *Canthium s.str.* and *Psydrax*; the arrangement of collenchyma in the midrib area which is distinctive in the *Psydrax* group; the shape of the vascular bundle of the midrib area and petiole which is probably the most useful character for identifying *Keetia gueinzii*; the extent of the xylem in the midrib area and petiole which assists in identifying or distinguishing between certain species; and the extent of cutinization of the outer and/or inner periclinal cell walls of the leaf epidermal cells which, for example, is of a characteristic shape in the midrib area for *C. suberosum*.

Moreover, it appears that leaf anatomy may be useful for diagnostic purposes irrespective of the stage of development of the leaf. For example, the type-B tanniniferous cells are easily recognizable in immature leaves where their pattern of distribution and abundance are similar to that of fully developed leaves. Type-B tanniniferous cells have essentially the same appearance in fresh and rehydrated material.

The present study has therefore shown that some of the uncertainty surrounding the generic and specific limits of *Canthium s.l.* in southern Africa can be resolved with the aid of leaf anatomical characters.

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